SALINITY TOLERANCE AND pH RANGE OF *CULEX FATIGANS* WIED., WITH NOTES ON THE ANAL PAPILLAE OF SALT-WATER MOSQUITOES.

By A. R. Woodhill, B.Sc.Agr., Dept. of Zoology, University of Sydney.

(One Text-figure.)

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Salinity Tolerance.

Introduction.

The mosquito *Culex fatigans* Wied. has usually been regarded as a typical freshwater species. Prior to 1937, the only reference to the possibility of it breeding in saline waters was made by Hamlyn-Harris (1928), who mentioned that in Queensland this mosquito rarely breeds in brackish water. Recently Doctors Wanson and Nicolay (1937) stated that at Banana, on the coast of the Belgian Congo, "the larval cycle is accomplished normally in concentrations of up to 30 grams of chloride per litre". Taking this figure as representing the total chlorides present, the salinity (S‰) expressed as total weight of salts in grams per 1,000 grams of water, would be in the vicinity of 33·5, i.e., only slightly lower than normal sea-water. Experiments previously carried out by the author (Woodhill, 1936) showed that larvae were rapidly killed by sea-water of S‰ 35·5, but no lower salinities were tested at that time. In view of the statement by Wanson and Nicolay, more detailed series of experiments were recently carried out at the University of Sydney.

Laboratory cultures derived from naturally-occurring eggs were reared in various dilutions of sea-water, using tap-water of S‰ 0·06 as a control. The salinity of the sea-water was determined by titration against "normal" sea-water, according to the method given by Harvey (1928), and the dilutions were again titrated before use. The general technique of breeding, with a constant food supply, was similar to that previously described for *Aëdes concolor* (Woodhill, 1936).

Laboratory Experiments.

In the first series, eggs were allowed to hatch in tap-water (S‰ 0·06), and the first stage larvae were transferred to water of the desired salinity within 12 hours of hatching. The results are given in Table I.

It will be seen from Table I that the larvae developed normally in S‰ 9, that there was a slight but significant* reduction in S‰ 10, while a heavy mortality with considerable individual variation took place in S‰ 11.

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* Assuming a binomial distribution the Standard Error is given by the formula

$$\text{S.E.} = \sqrt{p \times q \times N}$$

where p = the probability of adult emergence

$q = 1-p$ = probability of non-emergence

N = number of individuals

The Standard Error of a difference = $\sqrt{\text{SE}^2_{(1)} + \text{SE}^2_{(2)}}$

and if the difference between two means is greater than twice this figure the difference is assumed to be significant.

TABLE 1.

1st instar larvae of C. fatigans hatched in S‰ 0 and transferred within twelve hours to water of various salinities plus food. 20 larvae in each experiment. Constant temp. of 80° F.

No. of Experiment.	S‰.	No. of 4th Inst. Larvae.	No. of Pupae.	No. of Adults.	Mean per cent. Adults.
1	0	20	20	20	
2	..	18	18	18	
3	..	19	19	19	$97 \pm 1 \cdot 71$
4	..	20	20	20	
5	,,	20	20	20	
6	9	20	20	20	
7	..	20	20	20	
8	,,	20	20	20	$99 \pm 0 \cdot 995$
9	,,	20	19	19	
10	,,	20	20	20	
11	10	20	20	20	
12	,,	15	15	15	
13	,,	17	17	16	$86 \pm 3 \cdot 46$
14	,,	20	20	19	
15	,,	19	18	16	
16	11	10	10	10	
17	,,	3	3	2	
18	..	5	5	5	$30 \pm 4 \cdot 58$
19	..	9	9	9	
20	,,	4	4	4	
21	12	0	—	—	
22	,,	0	—	—	
23	,,	1	1	1	$1 \pm 0 \cdot 995$
24	,,	0	—	—	
25	,,	0	—	—	
26	13	0	—	—	
27	,,	0	—	—	
28	,,	0	—	—	0
29	,.	0	—	—	
30	,,	0	—	—	
31	30	0	—	—	
32	,,	0	—	—	
33	,,	0	—	—	0
34	,,	0	—	—	

Odd larvae may be able occasionally to develop in S‰ 12, while S‰ 13 is fatal to all larvae, death taking place within two days. In S‰ 30 the death of all larvae occurred within 8 hours. It is obvious that the critical point for 1st instar larvae is in the vicinity of S‰ 11.

In order to determine whether 4th instar larvae were any more resistant, bulk lots of larvae were bred through to the 4th instar in tap-water and then transferred to various salinities. The results are given in Table II.

TABLE II.

4th instar larvae of C. fatigans bred in S‰ 0 and transferred to water of various salinities plus food. 20 larvae in each experiment. Constant temp. 80° F.

No. of Experiment.	$S‰$.	No. of Pupae.	No. of Adults.	Mean per cent. Adults.
1	0	19	19	
2	,,	20	20	
3	,,	20	20	99 ± 0.995
4	,,	20	20	
5	,,	20	20	
6	9	20	20	
7	,,	20	20	
8	,,	18	18	98 ± 1.40
9	,,	20	20	
10	,,	20	20	
11	10	17	13	
12	,,	19	19	
13	,,	17	17	88 ± 3.25
14	,,	19	19	
15	,,	20	20	
16	11	13	9	
17	,,	11	11	
18	,,	10	8	49 ± 5.0
19	,,	11	11	
20	,,	10	10	
21	12	4	1	
22	,,	4	3	
23	,,	0	0	7 ± 2.55
24	,,	5	1	
25	,,	3	2	
26	13	0	—	
27	,,	0	—	
28	,,	0	—	0
29	,,	0	—	
30	,,	0	—	
31	30	0	—	
32	,,	0	—	
33	,,	0	—	0
34	,,	0	—	
35	,,	0	—	

From Table II it is evident that there is a slight increase in resistance to salinity in 4th instar larvae, as compared to 1st instar larvae, since there is a significant difference between the mean percentage surviving in each group in $S‰$ 11.

A series of experiments was then carried out to determine whether the salinity tolerance could be altered by gradually increasing the salinity of the water. The results are given in Table III.

TABLE III.

Larvae of C. fatigans *subjected to gradual increases in salinity.   20 larvae in each experiment plus food.   Constant temp. 80° F.*

| No. of Exp. | Salinity in which various instars were passed. Figures = $S‰$. | | | | No. of Pupae. | No. of Adults. | Mean per cent. Adults. |
	Eggs and 1st inst.	2nd inst.	3rd inst.	4th inst.			
1	9	10	11	11	15	15	
2	,,	.,	,,	.,	14	11	
3	,,	.,	,.	,,	16	12	64 ± 4·79
4	..	..	..	,,	14	12	
5	.,	,,	,,	,,	14	14	
6	9	10	11	12·5	6	4	
7	.,	.,	..	.,	6	5	
8	..	..	,,	.,	6	3	16 ± 3·66
9	..	..	,,	,,	4	1	
10	..	,.	,,	,,	5	3	
11	9	10	11	12·5→14	1	1	
12	.,	,,	,,	,, ,,	3	3	
13	..	..	.,	,, .,	4	4	17 ± 3·76
14	..	,,	..	,. .,	5	5	
15	..	..	.,	.. .,	4	4	
16	9	10	11	12·5→14→16	0	—	
17	,	.,	,,	,, ,, ,,	0	—	
18	,.	,,	,,	,, ,, ,,	0	—	0
19	,	..	,,	,, ,. ,,	0	—	
20	,,	.,	,.	. .. ,,	0	—	

It will be seen from Table III that some adaptation to increasing salinity takes place, since there is a significant difference between the number of adults produced in $S‰$ 11 in Table III as compared with Table II. In addition, 17% of adults were produced in $S‰$ 14 when the increase was gradual, whereas no adults were obtained from $S‰$ 13 when 4th instar larvae were transferred directly.

TABLE IV.

Pupae of C. fatigans *bred in tap-water ($S‰$ 0) and transferred to various salinities.   20 pupae in each experiment. Constant temp. 80° F.*

No. of Experiment.	$S‰$.	No. of Adults.	Mean per cent. Adults.
1	35·5	19	
2	,,	20	
3	..	18	97 ± 1·71
4	..	20	
5	..	20	
6	71·0	19	
7	.,	20	
8	..	20	99 ± 0·995
9	.,	20	
10	..	20	
11	105·0	17	
12	,.	18	86·6 ± 2·69
13	.,	17	

The effect of increased salinity on the pupae is, however, quite different. Larvae were bred through to the pupal stage in tap-water (S‰ 0) and transferred to various concentrations of sea-water as soon as the pupae were fully pigmented. The results are shown in Table IV.

It is obvious from Table IV that pupae can withstand an abrupt change from fresh water to water of approximately three times the concentration of normal sea-water and are quite independent of the osmotic pressure of the surrounding medium.

Finally, a few experiments were carried out to determine the effect of a solution of sodium chloride on the larvae. The results are shown in Table V.

TABLE V.

1st instar larvae of C. fatigans, *hatched in S‰ 0 and transferred within 12 hours. 20 larvae in each experiment. Constant temp. 80° F.*

No. of Experiment.	Solution.	No. of 4th Inst. Larvae.	No. of Pupae.	No. of Adults.	Mean per cent. Adults.
1	NaCl in dist. water, 10 gm. per litre.	0	—	—	
2	,,	0	—	—	0
3	,,	0	—	—	
4	,,	0	—	—	
5	Dist. water.	20	20	20	95
6	,,	19	18	18	

From Table V it will be noted that all larvae are killed by a solution of pure sodium chloride of a salinity of 10 gm. per litre, whereas they develop normally in a balanced solution of S‰ 10, i.e., diluted sea-water. This confirms the experiments carried out by previous workers on other species of mosquitoes.

Influence of Salinity Changes on Developmental Period.

In water of S‰ 0 to S‰ 10 at 80° F. the period from hatching to adult emergence varied from 8 to 12 days, and in S‰ 11 to S‰ 14 from 9 to 13 days. It is apparent, therefore, that increased salinity does not retard development to any great extent.
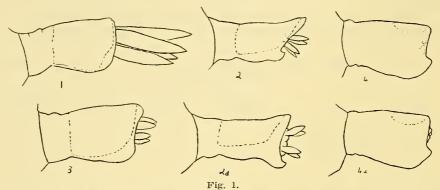
Discussion.

Taking the results of Tables I to V, it is found that the reaction of *Culex fatigans* to saline water is such as one would expect from a typical freshwater mosquito. The results agree in a general way with those of Wigglesworth (1933, *a* and *b*) for the larvae of *Aëdes* (*Stegomyia*) *aegypti*, but cannot be accurately compared since in those experiments the larvae were not in all cases bred through to the adult stage.

As shown by the same author, the critical point appears to be reached when the osmotic pressure of the environment becomes slightly greater than that of the haemolymph.

Changes in the walls of the anal papillae, similar to those described by Wigglesworth in *A. aegypti*, were observed in S‰ 11 to S‰ 15, with considerable individual variation. When 3rd instar larvae were transferred from S‰ 10 to S‰ 13·5 the anal papillae were frequently destroyed, and when the larvae were transferred back to S‰ 10, development to the adult stage took place without anal papillae.

Although some 3rd instar larvae can develop to the adult stage without anal papillae, the evidence so far available indicates that all those species normally found in salt water have anal papillae either reduced or absent.

The three species in the Sydney district which are frequently found in salt water, *Aëdes (Mucidus) alternans* West, *Aëdes (Ochlerotatus) vigilax* Sk., and *Aëdes (Pseudoskusea) concolor* Tayl., all have anal papillae reduced or absent, and this is also the case with a New Zealand salt-water species *Opifex fuscus* Hutton.

Fig. 1.

(1) *Culex fatigans*, (2) *Aëdes alternans*, normal appearance, (2a) *A. alternans* with rectum protruding, (3) *Aëdes vigilax*, (4) *Aëdes concolor*, normal appearance, (4a) *A. concolor*, with rectum protruding.

In Figure 1 the relative length of the anal papillae and the terminal segment of *C. fatigans* and the three salt-water species is shown. The appearance varies greatly according to whether the rectum is protruded or retracted. The structures previously termed rudimentary anal papillae in *A. concolor* are considered by the writer not to be homologous with the anal papillae but with internal rectal papillae. They are only evident externally when faeces are passed or when the larva is squeezed or preserved in strong fixative. Normally they are hidden within the anal opening. Similar structures, along with true anal papillae, can be seen in the figures of *A. alternans* (2 and 2a). Mosquito larvae when placed alive in strong fixative frequently protrude the rectum and lose the anal papillae, and these internal structures can then readily be mistaken for small anal papillae and have sometimes been figured as such.

The results of these experiments do not confirm those given by Wanson and Nicolay for *C. fatigans* in the Belgian Congo. If a biological race of *C. fatigans*, capable of breeding in salt water, exists there, one would expect the anal papillae to be very much reduced, as the result of a racial difference, or completely lacking owing to loss during the life of the individual. Wigglesworth (1938) has shown that even in water of S‰ 9 the anal papillae of *C. pipiens* are greatly reduced in size.

On the other hand, it would be possible to obtain adults of *C. fatigans* from natural waters of a very high salinity, provided that the influx of salt water took place after the pupae had developed.

The pH Range.
Introduction.

A considerable amount of work has been carried out on the pH range of *C. fatigans*, and this has been summarized by Senior White (1934). The results

from both field observations and laboratory experiments have given very conflicting results, some authors stating that acidity was fatal, while others found that the larvae were killed by a high alkalinity. In some laboratory experiments the acidity was produced by fermenting material such as banana pulp, while in others the chemicals used to control the pH were definite larvae poisons, and no attention was paid to the quantity or nature of the food available for the larvae. It seems obvious that factors other than the changed pH were operating in many of these experiments.

Laboratory Experiments.

In the following series of experiments the same standard food as previously used by the author (Woodhill, 1936) was supplied throughout in a measured quantity of water. The control series were put up in tap-water plus food, the pH of the mixture remaining between 6·4 and 6·8. The pH of this mixture was altered by the addition of small quantities of acetic acid or sodium hydroxide. It was found that the pH could readily be kept between 3·6 and 4·2 by the addition of 2·5 c.c. acetic acid (E) per litre, and between 3·4 and 3·6 by adding 5 c.c. per litre. As was to be expected, it was more difficult to maintain a high pH. When water containing 40 c.c. $\frac{N}{20}$ NaOH per litre was used, the pH varied from 10·6 to 7·6, even when changed twice weekly. In order to keep the pH above 9·0 it was found necessary to use 100 c.c. $\frac{N}{20}$ NaOH per litre, changing the water twice weekly.

The results of these experiments are shown in Table VI.

TABLE VI.

1st instar larvae of C. fatigans, hatched in tap-water and transferred within 12 hours. 20 larvae in each experiment. Constant temp. 80° F.

No. of Experiment.	Water.	No. of 4th Instar.	No. of Pupae.	No. of Adults.	Average per cent. Adults.
1	5 c.c. Acetic (E)	3	3	3	
2	per litre	4	4	4	21·25
3	pH range	7	7	7	
4	3·4 to 3·6	4	3	3	
5	2·5 c.c. Acetic (E)	20	20	20	
6	per litre	20	20	20	98·75
7	pH range	19	19	19	
8	3·6 to 4·2	20	20	20	
9	40 c.c. $\frac{N}{20}$ NaOH	20	19	19	
10	per litre	20	20	20	96·25
11	pH range	20	20	20	
12	> 10·6 to 7·6	20	19	18	
13	100 c.c. $\frac{N}{20}$ NaOH	19	19	19	
14	per litre	20	20	20	97·5
15	pH range	20	19	19	
16	> 10·6 to 9·0	20	20	20	
17	Tap-water	20	20	20	
18	pH range	20	20	20	98·75
19	6·4 to 6·8	20	20	20	
20		20	20	19	

It will be noted from Table VI that a normal percentage of adults developed from water with a pH varying from 3·6 to 4·2, 6·4 to 6·8, 7·6 to 10·6 and 9·0 to 10·6.

A significant reduction in number of adults took place in water of pH 3·4 to 3·6, but this result is not necessarily due to the direct effect of the low pH on the larvae, but may be due to its effect on the bacterial flora or to the direct effect of the excess acetic acid on the larvae. Senior White (1934) records larvae and pupae of *C. fatigans* in a tank of dilute hydrochloric acid with a pH of 1·6.

It is obvious therefore that any variation in pH between 4·2 and 9·0 does not prevent the development of *C. fatigans*.

Influence of pH Changes on Developmental Period.

The following gives the maximum and minimum periods from hatching to the emergence of adults at 80° F.

pH Range.	Period in Days.	
	Min.	Max.
3·4 to 3·6	15	17
3·6 to 4·2	11	16
6·4 to 6·8	8	12
7·6 to 10·6	11	16
9·0 to 10·6	11	16

It will be seen that a considerable change from the normal towards either acidity or alkalinity slightly retards the development, but from the data at present available it is not possible to say whether this is a direct effect on the larvae, or whether it acts indirectly by retarding the development of bacteria.

SUMMARY.

(1)   The statement by Doctors Wanson and Nicolay that *Culex fatigans* in the Belgian Congo develops normally in water containing 30 grams of chloride per litre has not been confirmed by experiments with the same species in Australia.

(2)   1st or 4th instar larvae, when transferred from tap-water direct to diluted sea-water, will not develop normally in concentrations greater than 10 gm. of salts per litre, and no adults develop in water containing 13 gm. of salts per litre.

(3)   When the salinity of the water is gradually raised, a few adults develop in water containing 14 gm. per litre.

(4)   Under no circumstances do any adults develop from larvae in water containing 16 gm. or more of salts per litre.

(5)   Larvae will not develop in water containing 10 gm. of NaCl per litre with no other salts present.

(6)   A comparison of the length of the anal papillae in relation to the terminal segment shows that all the species of mosquito larvae commonly found in salt or brackish water in Australia and New Zealand have anal papillae reduced or absent.

(7)   The structures formerly referred to as rudimentary anal papillae in *Aëdes (Pseudoskusea) concolor* Tayl. are considered to be homologous with internal rectal papillae.

(8)   Pupae are independent of the salinity of the water and will develop to adults in water containing 105 gm. of salts per litre.

(9)   Variation of the pH of the water from 6·8 to either 4·2 or 9·0 slightly retards the development of the larvae to the adult stage, but has no effect on the total number of adults produced.

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